Pharmacokinetics and Pharmacodynamics of Once-daily Prolonged-release Tacrolimus in Liver Transplant Recipients.

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Abstract

Purpose

There is limited published data regarding the pharmacokinetics (PK) and pharmacodynamics (PD) of prolonged-release tacrolimus (PRT) after liver transplant. We aimed to compare PK and PD of PRT in early and stable liver transplant recipients by developing a population PK model of PRT and investigating the profile of calcineurin activity (CNA) in the peripheral blood mononuclear cells.

Methods

A conversion from twice-daily immediate-release tacrolimus (IRT) to once-daily PRT based on one-to-one daily dose was performed at day 7 (D7) and D90 post-transplantation in groups A (n = 12) and B (n = 12), respectively. Extensive PK samplings including whole blood tacrolimus (TAC) concentration and CNA assessment were performed at D14 and D104 in groups A and B, respectively. TAC concentration-time data (n = 221) were analyzed using non-linear mixed effects modeling.

Findings

A two-compartment model with linear elimination and a delayed first order absorption characterized by two transit compartments best described PK data. Model-predicted dose-normalized (6.0 mg/day) area under the TAC concentration-time curve over the dosing interval (AUC_{TAC}) in groups A and B were similar (geometric mean 235.6 ng/mL.h [CI95% = 139.6 – 598.7] vs 224.6 ng/mL.h [117.6 – 421.5], respectively, p = 0.94). Area under the CNA versus time curve over the dosing interval (AUC_{CNA}) were not different between both groups (4897 ± 3437 and 4079 ± 1008 pmol/min/10^6 cells, respectively, p = 0.50). In group A, trough CNA at D14 post-transplantation was statistically higher than that measured just before the switch to PRT (i.e D7 post-transplantation) (198 ± 92 vs 124 ± 72 pmol/min/10^6cells, n = 8, respectively, p = 0.048), while no statistical difference in TAC concentration was observed (p = 0.11). In group B, no statistical difference between D90 and D104 was observed in either trough CNA (149 ± 78 vs 172 ± 82 pmol/min/10^6 cells, respectively, n = 6, p = 0.18) or TAC concentration (p = 0.17). No graft rejection was observed in either of the groups.

Implications

This study suggests that one-to-one dosage conversion to once-daily PRT during the early post-transplantation period could result in significant CNA variations but without causing graft rejection. Further investigations in larger cohorts are warranted to confirm these results.

Study registry identification number: ClinicalTrials.gov Registration identification NCT02105155

Keywords: liver transplantation; prolonged-release tacrolimus; pharmacokinetics; calcineurin activity

1. Introduction

Tacrolimus (TAC) is a key immunosuppressive agent for the prevention and treatment of allograft rejection in liver transplantation [1]. TAC binds with high affinity to FK-binding protein 12 [2]. The drug-receptor complex specifically and competitively binds to and inhibits calcineurin, a calcium- and calmodulin-dependent phosphatase. This process inhibits the translocation of a family of transcription factors (NF-AT), leading to reduced transcriptional activation of cytokine genes such as interleukin (IL)-2 and thereby to a reduction of T-cell proliferation [3]. TAC has a narrow therapeutic range and a significant between-subject variability (BSV), and thus a close monitoring of whole blood trough concentrations is required to avoid under- or over-exposure [4]. Hence, therapeutic drug monitoring of TAC in liver transplant recipients is the benchmark method in this indication [1]. However, some liver transplant recipients with sufficient exposure to TAC nonetheless experience graft rejection [5,6], suggesting that whole blood trough concentration may not be the most appropriate surrogate marker of pharmacodynamics (PD) in these patients. Different approaches such as evaluation of TAC intracellular concentration in peripheral blood mononuclear cells (PBMC) [7] or calcineurin activity (CNA) in PBMC [6–9] could be helpful to overcome this issue in those patients. However, they are not currently used for the clinical management of liver transplant recipients in daily clinical practice.

Liver transplant recipients are usually treated with twice-daily immediate-release tacrolimus (IRT) (Prograf®). Non-adherence to treatment has been found to be a significant factor associated with graft rejection and graft loss [10]. A once-daily prolonged-release tacrolimus (PRT) (Advagraf®) has been developed to improve treatment adherence. The phase III trial conducted in *de novo* liver transplant recipients showed that both efficacy and safety profiles were similar between twice-daily IRT and once-daily PRT [11]. The twice-daily dosage of IRT usually shifts to once-daily PRT based on a one-to-one conversion (i.e. same daily dose for IRT and PRT). The narrow therapeutic range and the significant BSV in the pharmacokinetics (PK) of TAC, could result in significant variations in PD in some patients, possibly leading to acute graft rejection within the early post-transplantation period. In this context, exploring both PK and PD of once-daily PRT at the time of conversion becomes mandatory. However, the PK data of once-daily PRT in liver transplant recipients are very sparse. A single population PK study was conducted to investigate once-daily PRT PK in

stable liver transplant recipients [12], while another study using a standard non-compartmental approach characterized its PK during the early post-transplantation period [13]. In this context, a population PK study including data from the early and late post-transplantation period could be interesting to better characterize the PK/PD relationship of once-daily PRT in liver transplant recipients. Finally, as far as we know, the profile of CNA has not been investigated in PBMC from liver transplant recipients treated with once-daily PRT.

The aim of this study was to describe the PK of once-daily PRT using a population approach and to characterize the CNA profile in PBMC in liver transplant recipients treated with once-daily PRT and included in the CONVERSION® trial.

2. Patients and Methods

Study population and treatment

The CONVERSION® trial (ClinicalTrials.gov Registration identification NCT02105155) is a prospective, randomized, multicenter trial aiming to prove the non-inferiority of the early conversion from IRT to PRT versus the conversion at three months after liver transplantation. Eligible patients (>18 years) underwent liver transplantation at day 1 (D1) and started treatment with IRT (Prograf®). A conversion from IRT to PRT (Advagraf®) was performed at D7 and D90 after transplantation in groups A and B, respectively (Figure 1). The dosage of twice-daily IRT shifted to once-daily PRT based on a one-to-one conversion (i.e same daily dose for IRT and PRT). After conversion, daily dosing was adjusted according to TAC whole blood trough concentration with a therapeutic range of 6 – 10 ng/mL [1]. All patients provided written informed consent. The protocol was approved by the Committee for the Protection of Persons and the French National Agency for Medicines and Health Products Safety.

Two hundred and fifty liver transplant recipients were supposed to be included in the CONVERSION® trial, and 40 of them in the PK/PD study (n = 20 in each group). However, only 90 patients were included in the CONVERSION® trial because of numerous simultaneous clinical trials. Furthermore, many patients refused to participate in the PK/PD study because of the lack of personal gain. In this context, PK and PD data come from 24 patients included in the CONVERSION® trial.

PK data collection

Extensive PK sampling was performed at D14 post-transplantation (i.e. at D7 post-conversion) in group A and at D104 post-transplantation (i.e. at D14 post-conversion) in group B (Figure 1). Blood samples (7 mL) were drawn before next administration (at trough), 0.33, 0.66, 1, 2, 3, 4, 6, 8 and 24 hours after drug intake. Blood samples were also collected right before next drug intake (trough concentration) at D5, D7, D14, D30, D90 and D180 post-transplantation in group A and at D90, D104 and D180 post-transplantation in group B (Figure 1). Whole blood TAC concentrations were assayed using an ECLIA method [14] on Cobas 8000 (Roche Diagnostics, Meylan, France). The calibration range of the ECLIA method was 1 – 40 ng/mL with a limit of detection of 0.5 ng/mL. The intermediate precision and

accuracy of the ECLIA method were below 8.1% and 5.1%, respectively, at three levels of concentrations (2.5, 10.4 and 19.8 ng/mL) [14]. The accuracy of our method was ensured by our participation in the TAC Proficiency Testing Scheme provided by the Cardiac and Vascular Sciences Analytic Unit of St. George's Hospital Medical School (D. Holt, London, United Kingdom).

At each follow-up visit, body composition and biological parameters were collected: body weight (BW), lean body mass (LBM), hematocrit (HT), glomerular filtration rate (GFR) estimated by Cockcroft-Gault formula, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), bilirubin (BIL). LBM was estimated according to the McLeay *et al.* formula [15].

Calcineurin activity in PBMC

Trough CNA in PBMC (just before drug intake) was assayed immediately before the switch to PRT (i.e. at D7 and D90 post-transplant for groups A and B, respectively, Figure 1). Furthermore, CNA was assayed on the blood samples from extensive PK sampling (D14 for group A and D104 for group B) before next administration (at trough), 0.33, 0.66, 1, 2, 3, 4, 6, 8 and 24 hours after drug intake. For each blood sample, PBMC isolation was performed within 24 hours after blood collection [16]. First, granulocyte depletion was performed to prevent the influence of granulocytes on CNA [17]. For this purpose, the RosettSep® kit was used according to the manufacturer's instructions (StemCell Technologies, Grenoble, France). Second, PBMC were isolated by Ficoll density-gradient centrifugation (Unisep Ficoll-tubes, Abcys, Jerusalem, Israel), then washed and counted with Xn-9000 (Sysmex, Villepinte, France). Each sample including 10^6 PBMC was dried and frozen at -80°C up to analysis. CNA assay was run in duplicate as previously described [16]. Briefly, PBMC lysates were incubated for 15 minutes at 30°C in analysis buffer including 50 mM Tris-HCl, pH 7.0, 0.1 M Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 0.5 mM dithiothreitol, 1 mM $MnCl_2$, 0.3 mg/mL bovine serum albumin, 0.1 mM EGTA, 1 mM $CaCl_2$, 0.1 µM calmodulin and 500 nM okadaic acid. The reaction was initiated by adding a 19 amino-acid phosphopeptide (DLDVPIPGRFDRRVSVAAE, Bachem, Voisin, France). Aliquots were sampled at 5 and 10 minutes. The reaction was stopped with 0.5% perchloric acid. Dephosphorylated peptide concentrations were determined using high-performance liquid chromatography

coupled with UV detection. The chromatography system consisted of Dionex Ultimate 300 equipped with a gradient pump with degas option and gradient mixer, a UV-visible detector, an autosampler, and a Chromeleon® chromatography workstation (Dionex Corporation, Sunnyvale, CA, USA). The within-day precision of this method was 13.3% including all the steps from blood collection to CNA assay [16]. CNA was expressed as picomole of formed dephosphorylated peptide per minute per 10^6 PBMC (pmol/min/10^6 cells).

Non-compartmental Pharmacokinetic Analysis

Whole blood concentrations of TAC from extensive PK sampling were used to calculate the area under the TAC concentration-time curve over the dosing interval (AUC_{TAC}) using the trapezoidal rule.

Population Pharmacokinetic Analysis

TAC concentration-time data were analyzed by nonlinear mixed effects modeling using NONMEM® software (version 7.4, ICON Development Solutions, Ellicott City, MD, USA) with Piraña® (version 2.9.7) and PsN toolkit (version 4.7.0). Analyses were carried out with first order conditional estimation method with interaction (FOCE-I). Data processing and plots were performed in R (version 3.4.2). Several structural models were used to fit the concentration-time data. First, one and two compartment models with first order absorption and elimination were tested. Since TAC was administered as a prolonged-release formulation, a first order process with either a lag time or transit compartments with an identical transfer rate constant (k_{tr}) were tested to account for the delay in the absorption phase. The inclusion of BSV and between-occasion variability (BOV) defined as $OCC_1 \leq$ D28 and $OCC_2 >$ D28 for group A and $OCC_1 \leq$ D105 and $OCC_2 >$ D105 for group B was tested on all PK parameters according to an exponential model:

$$\Theta_i = \Theta_\mu \cdot \exp(\eta_i + \eta_{1i}OCC_1 + \eta_{2i}OCC_2)$$

where Θ_i is the estimate of the parameter for the *i*th subject, Θ_μ is the population mean estimate of the PK parameter, η_i is the deviation from the mean for the *i*th subject with zero mean and variance ω^2, η_{1i} and η_{2i} is the deviation from the mean for the first (OCC_1) and second (OCC_2) occasion for the *i*th subject, respectively. Correlation between η of PK parameters was tested using a ω block structure. The residual unexplained variability was described using a proportional error model. Model selection was based on the objective

function value (OFV = −2loglikelihood), using the likelihood ratio test to test for significant differences in goodness-of-fit (GOF) between nested models. A drop of at least 3.84 (χ^2 test, α = 5%, degree of freedom = 1) between hierarchical models was considered statistically significant. Additionally, the plausibility of parameter estimates with their precision (expressed by relative standard error, %RSE), η-shrinkage value and model stability were considered.

Covariate analysis

The individual parameter estimates of the base model were used to investigate the correlations with biological and demographic variables. The following covariates were tested for their influence on clearance (CL): age, sex (0 for male and 1 for female), BW, LBM, HT, GFR, AST, ALT, ALB, BIL and study group (GRP). As PK data come from a large time period, different values of a covariate for the same patient were included in the data. Continuous covariates were tested according to the linear function:

$$CL = \Theta_{CL} \times (1 + \Theta_{cov} \times (cov - cov_{mean}))$$

where Θ_{CL} is the typical value of CL in the population, *cov* is the individual covariate value, cov_{mean} is the mean value of a covariate in the studied population, Θ_{cov} is the fractional change in CL from the mean value of the covariate. Categorical covariates (sex, study group) were tested according to the following equation:

$$CL = \Theta_{CL} \times \Theta_{cov}^{COV}$$

where Θ_{cov} is the estimated influential factor for a covariate and *cov* is 1 or 0. In the forward procedure, covariates were tested one by one and a covariate was considered significantly associated with a PK parameter if its inclusion resulted in a drop in OFV of at least 3.84 points (χ^2 test, α = 5%, df = 1). In the backward procedure, a full covariate model including the covariates significant in the forward procedure was built. A covariate remained in the final model if its removal resulted in an increase of at least 6.63 points (χ^2 test, α = 1%, df = 1) compared to the full covariate model.

Model evaluation

Diagnostic plots including population predictions (PRED) versus observed concentrations (DV), individual predictions (IPRED) versus DV, conditional weighted residuals (CWRES)

versus PRED and time after dose were generated. Since patients were treated with different doses of TAC, the model validation was performed with a prediction-corrected visual predictive check (pcVPC) based on 1000 replicates of the original data set and presented as concentrations versus time after dose and stratified on study group to facilitate interpretation. Finally, 500 bootstrap analyses with resampling using the final model were performed.

Analysis of the individual PK parameters

The individual CL values obtained in NONMEM were used to calculate AUC_{TAC} according to the following formula in the model input file:

$$AUC_{ij} = DOSE_i \times F/CL_{ij}$$

where AUC_{ij} is the area under the concentration-time curve over the dosing interval for the *i*th subject and *j*th occasion, $DOSE_i$ is the administered dose for the *i*th subject, CL_{ij} is the individual clearance value for the *i*th subject and *j*th occasion and *F* is the oral bioavailability of TAC (fixed in the model to 0.23 based on the literature) [18].

Statistical Analysis

The demographic and biological characteristics of the study cohort are presented as median [interquartile range]. PK data are expressed as geometric mean [95% confidence interval, CI95%] and PD data are expressed as mean ± SD. The individual AUC_{TAC} values obtained by non-compartmental analysis and population approach were normalized by the median daily dose which was administered prior to extensive PK sampling. Individual AUC_{TAC} obtained in the non-compartmental analysis were compared with model-predicted AUC_{TAC} for group A and B using non-parametric Wilcoxon paired sample test. AUC_{TAC} obtained by both non-compartmental analysis and population approach were compared between group A and B using a Wilcoxon unpaired samples test. Since the number of patients per each study group is low, the ratio of the geometric means of AUC_{TAC} group A over AUC_{TAC} group B as well as its CI90% was calculated in addition to the non-parametric statistical tests to compare AUC_{TAC} between groups A and B.

From data of extensive PK sampling, individual 24-hour area under the calcineurin activity versus time curve (AUC_{CNA}) was calculated using the trapezoidal rule. Only PD data from

extensive PK sampling were used to investigate the PK/PD relationship. The AUC_{CNA} were compared between groups A and B using a Wilcoxon unpaired samples test. The relationship between AUC_{TAC} and AUC_{CNA} was tested using Spearman's correlation test. All tests were two-sided, and they were considered significant when p-values were <0.05. Computations were performed using R software and SAS V9 statistical package (SAS institute, Cary, NC, USA).

3. Results

Patients and TAC concentrations

The baseline demographic and biological characteristics of 24 patients (*n* = 12 patients in each group) included in the study are summarized in Table 1. Overall, 221 blood samples including those from therapeutic drug monitoring were available for the PK analysis. The median number of measurements per individual was 11 (range 1 – 13). The sampling time was in the range 0.1 – 27 h after drug intake. Four patients who did not have extensive PK sampling (*n* = 1 in group A and *n* = 3 in group B) withdrew their informed consent on the day of the analysis as they did not understand that a part of the study included several blood samples drawn throughout the day and required them to stay for a longer time in the medical department. For the remaining patients (*n* = 20), extensive PK sampling was performed at median 14 days (range 13 – 21) and 104 days (95 – 109) after transplantation in groups A and B, respectively. Figure 2 presents TAC concentrations versus time after dose at D14 (*n* = 11) and D104 (*n* = 9) for groups A and B, respectively (data from extensive PK sampling only).

Non-compartmental Pharmacokinetic Analysis

AUC_{TAC} values were calculated using trapezoidal rule for the 20 patients (*n* = 11 and *n* = 9 for groups A and B, respectively) for which extensive PK data were available. The absolute AUC_{TAC} means obtained by the non-compartmental analysis were similar between groups A and B (251.3 ng/mL.h [CI95% = 108.5 – 460.7] and 200.7 ng/mL.h [CI95% = 126.0 – 302.2], respectively, *p* = 0.17).

At the time of extensive PK sampling, the median dose of PRT was 7.0 mg/day and 5.0 mg/day in groups A and B, respectively, whereas median dose was 6.0 mg/day regardless of study group. The geometric means of dose-normalized AUC_{TAC} (6.0 mg/day) were similar in groups A and B (234.5 ng/ml.h [CI95% = 130.3 – 670.6] and 231.0 ng/ml.h [CI95% = 120.2 – 433.4], respectively). The ratio of the geometric means of $AUC_{TAC\ group\ A}$ over $AUC_{TAC\ group\ B}$ was 1.01 [CI90% = 0.66 – 1.56] (Table 2). The dose-normalized AUC_{TAC} obtained by non-compartmental analysis were not statistically different between groups A and B (*p* = 0.77).

Population Pharmacokinetic Analysis

TAC concentration-time data were described by a two-compartment model with linear elimination and a delayed first order absorption characterized by two transit compartments with an identical k_{tr}. Addition of transit compartments to describe the absorption phase resulted in a significant improvement of the model fit: one transit compartment dropped OFV by 14 points and two transit compartments by 25 points compared to the model without delayed absorption. Further addition of a third transit compartment did not improve the model fit. The PK parameters of the final model were: k_{tr}, clearance (CL), volume of distribution of the central compartment (V_c), inter-compartmental clearance (Q), volume of distribution of the peripheral compartment (V_p). The bioavailability (F) of TAC was fixed to the value previously reported in the literature (F = 0.23) [18]. Therefore, the PK parameters (CL, V_c, Q, V_p) were reported as absolute values. BSV was included on k_{tr}, CL and Q. BSV could not be reliably estimated on V_c and V_p and inclusion of BSV on F did not improve the model fit, thus BSV was fixed to zero for these three parameters. The addition of covariance between η of the PK parameters did not improve the model fit. Finally, inclusion of BOV on CL resulted in a drop of 86 points in OFV and decreased the residual variability from 27.8% to 19.8%.

Covariate analysis

The covariate analysis was performed on CL only as η_{ktr} showed significant deviation from a normal distribution (Shapiro-Wilk test, p = 0.02) and η_Q was associated with shrinkage of 35%. The correlation plots between individual CL of OCC_1 and OCC_2 and continuous covariates are presented in Supplementary Figure 1. The lack of influence of sex and GRP on CL is presented in Supplementary Figure 2. In the forward analysis, none of the tested covariates was significantly associated with CL (Supplementary Table 1) thus the final model did not include covariates. The estimates of the final model with corresponding %RSE are presented in Table 3.

Evaluation of the final model

GOF plots depicted in Figure 3 show no major bias of the model based on IPRED vs DV plot whereas CWRES vs PRED and time after dose were homogeneously distributed around the zero line although a slight bias at higher PRED values was observed. The pcVPC showed that

the 5th, 95th percentiles and the median of the simulated data are in good agreement with the 5th and 95th percentiles and the median of the observed concentrations for both groups A and B (Figure 4). Finally, the mean estimates of the PK parameters from 500 bootstrap analyses are in accordance with those estimated using the original data set (Table 3).

Analysis of individual PK parameters

Model-predicted absolute AUC_{TAC} at extensive PK sampling (corresponding to OCC_1 for both group A and B) was no statistically different between group A and B (252.4 ng/mL.h [CI95% = 111.3 – 510.5] vs 195.2 ng/mL.h [CI95% = 124.9 – 302.1], respectively, p = 0.17, n = 20). Table 2 presents model-predicted geometric means of AUC_{TAC} normalized for a median dose of 6.0 mg/day. Dose-normalized AUC_{TAC} were not statistically different between groups A and B (235.6 ng/mL.h [CI95% = 139.6 – 598.7] and 224.6 ng/mL.h [CI95% = 117.6 – 421.5] ng/mL.h, respectively, p = 0.94) and the ratio of the geometric means of $AUC_{TAC\ group\ A}$ over $AUC_{TAC\ group\ B}$ was 1.05 [CI90% = 0.70 – 1.57] (Table 2). Finally, the comparison of AUC_{TAC} obtained either by non-compartmental analysis or by population approach showed that both values were similar (p = 0.90 and p = 0.25 for groups A and B, respectively) further validating our PK model.

PRT pharmacodynamics

Figure 5 presents individual CNA profile (log scale) over the dosing interval at D14 for group A (n = 11) and D104 for group B (n = 9). The AUC_{CNA} means were not statistically different between groups A and B (4897 ± 3437 and 4079 ± 1008 pmol/min/10^6 cells, Wilcoxon unpaired t-test p = 0.50). However, a larger BSV in AUC_{CNA} was observed in group A (70.2 vs 24.7% for groups A and B, respectively). No relationship was found between AUC_{CNA} and either model-predicted absolute AUC_{TAC} (rho coefficient, ρ = 0.26, [CI95% = -0.20; 0.63]; p = 0.25; Figure 6) or TAC whole blood trough concentration (rho coefficient, ρ = 0.20, [CI95% = -0.27; 0.59], p = 0.39). The mean trough CNA activity (just prior TAC intake) at D14 post-transplantation in group A was statistically higher than that measured just before the switch to PRT (i.e. D7 post-transplantation) (198 ± 92 vs 124 ± 72 pmol/min/10^6cells, n = 8, respectively; paired t-test, p = 0.048), while no statistical difference was observed for TAC whole blood trough concentration (6.9 ± 2.3 vs 10.1 ± 5.4 ng/mL, respectively; paired t-test, p = 0.11). Finally, no statistical difference between D90 and D104 was observed for either

trough CNA (149 ± 78 vs 172 ± 82 pmol/min/10^6cells, n = 6; paired t-test, p = 0.18) or TAC whole blood trough concentration (8.8 ± 4.6 vs 5.9 ± 2.1 ng/mL, respectively; paired t-test, p = 0.17). Finally, no graft rejection was observed in either group.

4. Discussion

PRT (Advagraf®) is EMA-approved for use in the context of liver transplants. However, there is limited published data regarding the PK and PD of PRT in this indication. As far as we know, the present study is the first to assess the PK of PRT within the early and late post-transplantation periods using a population approach. Furthermore, it provides new insights about the profile of CNA in PBMC from liver transplant recipients treated with PRT.

In the population PK analysis, blood concentration-time data of once-daily PRT were described by a two-compartment model with delayed absorption characterized by two transit compartments. This is consistent with a previous population PK study reported by Moes *et al*. in which a two-compartment model with three transit compartments was used to characterize the PK of once-daily PRT in 66 stable liver transplant recipients [12]. The mean estimate of CL in our analysis was 5.1 L/h (BSV = 34.7%) which is close to the value reported by Moes *et al*. (4.77 L/h, BSV = 45.4%). The analysis of the demographic and biological covariates on CL did not allow us to identify any significant correlations. This may be due to small sample size and the small dispersion of the covariates in our study. Nevertheless, in stable liver transplant recipients treated with PRT, Moes *et al.* did not report any significant influence of the covariates which we tested on total CL [12].

It has been reported that the expression of *CYP3A5*1*, both in donor and receiver of a liver transplant, significantly increases CL of TAC in patients treated with PRT [12]. Other studies conducted in kidney transplant recipients treated with PRT also reported the influence of *CYP3A5*1* on CL [19,20]. We could not confirm or contradict these results because pharmacogenetic data were not available in our study. It was decided not to conduct an analysis of *CYP3A5*1* genotype in the CONVERSION study because the frequency of *CYP3A5*1* genotype in the French population is low (13%) [21] and as the study included a small number of patients, the statistical power would not have been sufficient to draw any firm conclusion. Similarly, using a mixture model in the PK population analysis to identify the subpopulation carrying the *CYP3A5*1* allele would not have been possible. Regarding the genetic polymorphisms of drug transporters such as MDR1, although its influence on TAC PK has been reported, the results still remain controversial[22]. In the same way as for the *CYP3A5*1* genotype, our study could not contribute any results regarding the impact of

genetic polymorphisms of drug transporters on TAC PK because of the lack of statistical power.

To further evaluate the validity of our model, the individual AUC_{TAC} obtained using a population approach were compared with those obtained using a non-compartmental analysis with data from extensive PK sampling. AUC_{TAC} means of groups A and B obtained using either a non-compartmental or a population approach were not statistically different (p = 0.90 and p = 0.25 for groups A and B, respectively). Furthermore, comparison of AUC_{TAC} values obtained by both approaches showed no statistical differences between groups A and B (p = 0.77 and p = 0.94, respectively). Finally, the geometric means of model-predicted dose-normalized AUC_{TAC} in our study (235.6 ng/mL.h [CI95% = 139.6 – 598.7] and 224.6 ng/mL.h [CI95% = 117.6 – 421.5] for groups A and B, respectively, normalized to median dose of 6.0 mg/day) are close to those previously reported in liver transplant recipients obtained using a non-compartmental approach [23]. Indeed, Florman *et al.* reported a mean AUC_{TAC} of 184 ± 63 ng.h/mL at day 28 post-transplantation in liver transplant recipients treated with PRT (mean dose of 5.2 mg/day). Taken together, these results suggest that the developed model satisfyingly describes the TAC concentration-time data. However, the limitation of our PK analysis is the small number of patients. Therefore, our results are not conclusive and need to be confirmed in larger cohorts. Moreover, some individual PK profiles in our study show a second peak of absorption. This was previously observed in liver and kidney transplant recipients treated with a different PRT formulation (Envarsus®)[24,25] and was described by a double-gamma absorption model. In our analysis, the attempts to describe the second absorption peak did not give a reliable estimation of the PK parameters probably due to an insufficient number of samples in the absorption phase or the fact that it was only observed in some patients. In addition, the low number of PK samples in the absorption and distribution phases might be the reason for high BSV on k_{tr} and Q. Although we analyzed the PK data with a model which did not account for the second peak of absorption, the comparison of AUC_{TAC} values obtained with the non-compartmental approach and predicted by the PK model were in good agreement for both study groups which shows that our model accurately described the data.

CNA is a surrogate marker of TAC PD. Different PK/PD studies conducted in liver transplant recipients have suggested that assessment of CNA within the early post-transplantation period could be helpful to predict acute graft rejection in patients well exposed to TAC [6,7]. In

the present study, no relationship was found between AUC_{TAC} and AUC_{CNA} values regardless of the moment of conversion from IRT to PRT, as previously reported [6–8]. Different factors such as the amount of cytosolic FKBP12 [26] and FKBP13, FKBP51 acting as a reservoir [2], the genetic polymorphism of the calcineurin catalytic subunit α [27,28] and the etiology of liver disease before transplant [29] might significantly influence the CNA in PBMC regardless of the whole blood TAC concentration. Besides, Lemaitre *et al.* showed that CNA in PBMC was not further associated to intracellular concentration of TAC in liver transplant recipients[7], which supports our result. The BSV in AUC_{CNA} for group A is in accordance with that reported at D7 and D14 post-transplantation in liver transplant recipients treated with twice-daily IRT [6–8]. However, it was 3-fold higher compared to the BSV in AUC_{CNA} for group B (70.2 vs 24.7%, respectively) while AUC_{CNA} means were not statistically different in both groups. In addition, absolute AUC_{TAC} values were similar between groups A and B (p = 0.17) which altogether suggests that factors other than drug exposure contribute to this variability. Although patients' characteristics regarding immunophilins (FKBP12, 13 and 51) were probably different between both groups, the magnitude of immune response during the early post-transplantation period might also contribute to the large BSV in AUC_{CNA}. Besides, our study shows that the conversion from IRT to PRT in a 1:1 ratio based on total mg/day dose could also contribute to this variability. Interestingly, trough CNA at D14 in group A was statistically higher than that measured just before the switch to PRT (p = 0.048), while no difference in TAC whole blood trough concentration was observed. Furthermore, neither trough CNA nor TAC whole blood trough concentration at D90 and D104 was different in group B. Finally, no graft rejection was observed in our PK/PD study regardless of study group. Although the number of patients was limited, these results suggest that the conversion from IRT to PRT during the early post-transplantation period could modify PD profile of calcineurin without causing graft rejection. Further investigations with a larger cohort of patients should be conducted to confirm this result.

In conclusion, we have developed a population PK model for PRT in order to evaluate the PK/PD relationship for TAC in early and stable liver transplant recipients. The results suggest that one-to-one dosage conversion from twice-daily IRT to once-daily PRT during the early post-transplantation period could modify CNA in PBMC which might not be related to TAC PK. The advantage of our study is the PK and PD comparison between early and stable transplant recipients. Using both a non-compartmental analysis and a population approach,

we showed that the mean AUC_{TAC} values between group A and B were not statistically significantly different. Therefore, the model we have developed can be used to predict TAC whole blood concentrations in liver transplant recipients under the same conditions and dosing regimen as specified in our study. However, as the sample size in our study is low, our results should first be confirmed in larger cohorts.

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Figure Legends

Figure 1. Design of the pharmacokinetic/pharmacodynamic study in the CONVERSION® trial.

Figure 2 Individual pharmacokinetic profiles of once-daily prolonged-release tacrolimus from extensive sampling day, corresponding to day 14 for group A (*n* = 11) and day 104 for group B (*n* = 9).

Figure 3. Goodness-of-fit plots of the final model. (PRED, population predictions, IPRED, individual predictions, DV, observed concentrations, CWRES, conditional weighted residuals).

Figure 4. Prediction-corrected visual predictive check stratified on study group based on 1000 replicates of the original data set using the final model. *Blue lines* represent the 5th and 95th percentiles of the observed concentrations, *red line* represents the median of the observed concentrations, *blue areas* represent 95% confidence intervals around 5th and 95th percentiles of the simulated concentrations, *red area* represents 95% confidence interval around the median of the simulated concentrations and *black points* represent observed concentrations.

Figure 5. Individual CNA profile over the dosing interval at day 14 for group A (*n* = 11) and day 104 for group B (*n* = 9).

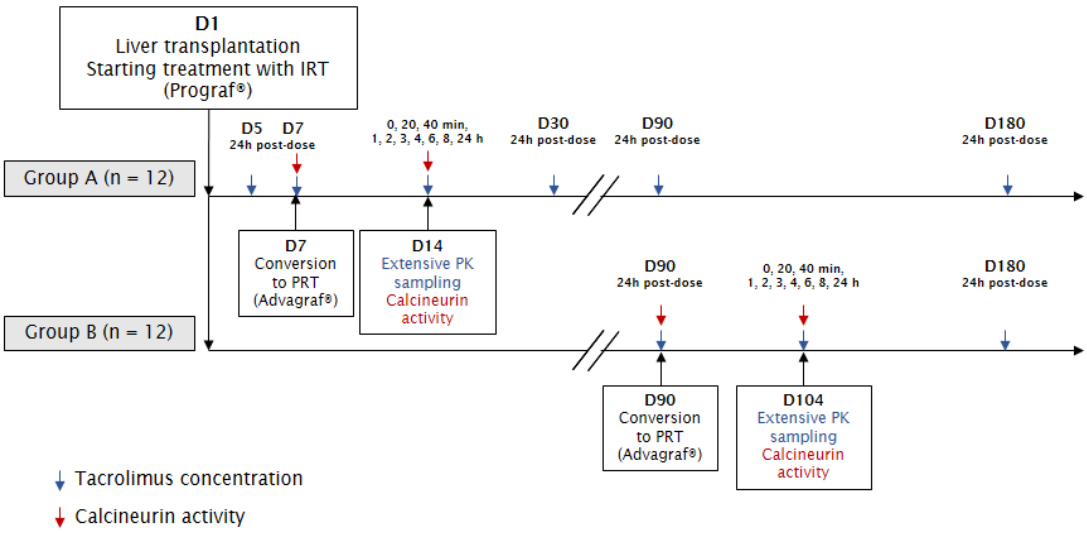
Figure 6. Relationship between area under the tacrolimus concentration-time curve over the dosing interval (AUC_{TAC}) and 24-hour area under the calcineurin activity curve (AUC_{CNA}) in liver transplant recipients treated with once-daily prolonged-release tacrolimus. Calcineurin activity (CNA) is expressed for 10^6 cells.

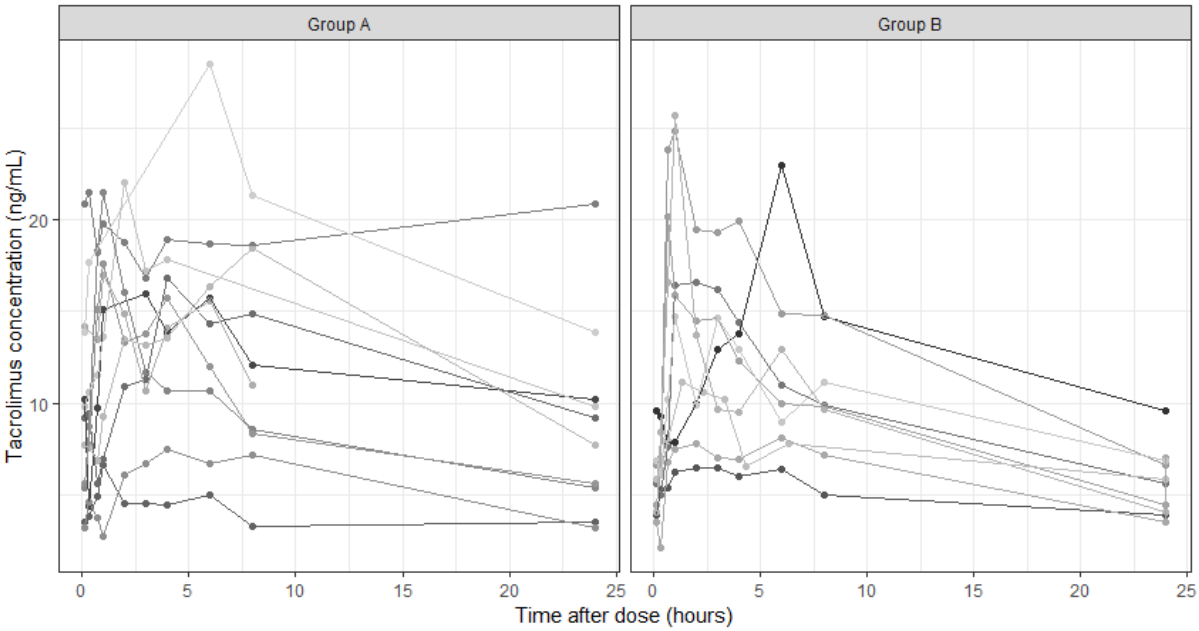
Supplementary Material

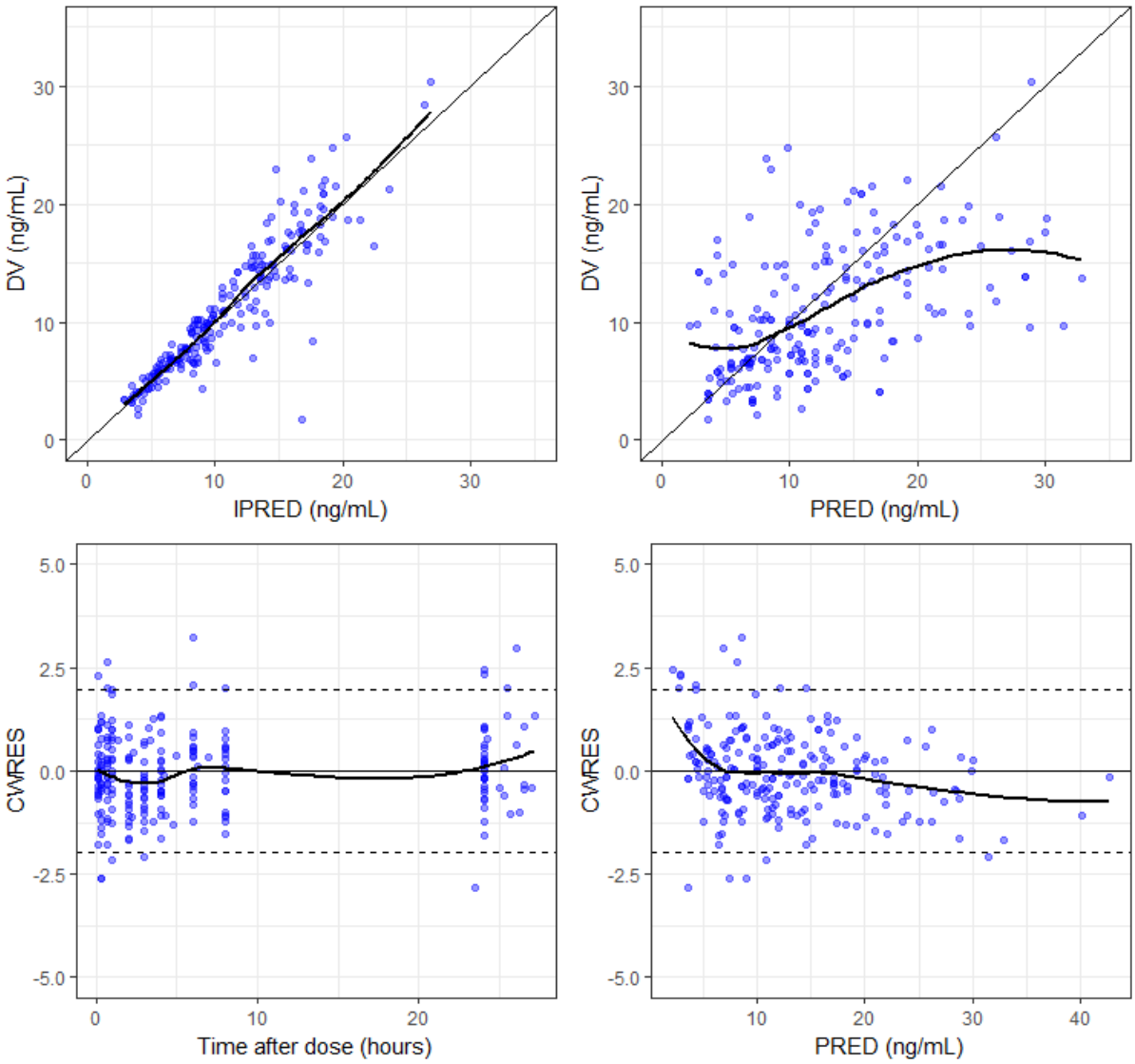
Supplementary Table 1 Results of the covariate analysis using the base model (forward step).

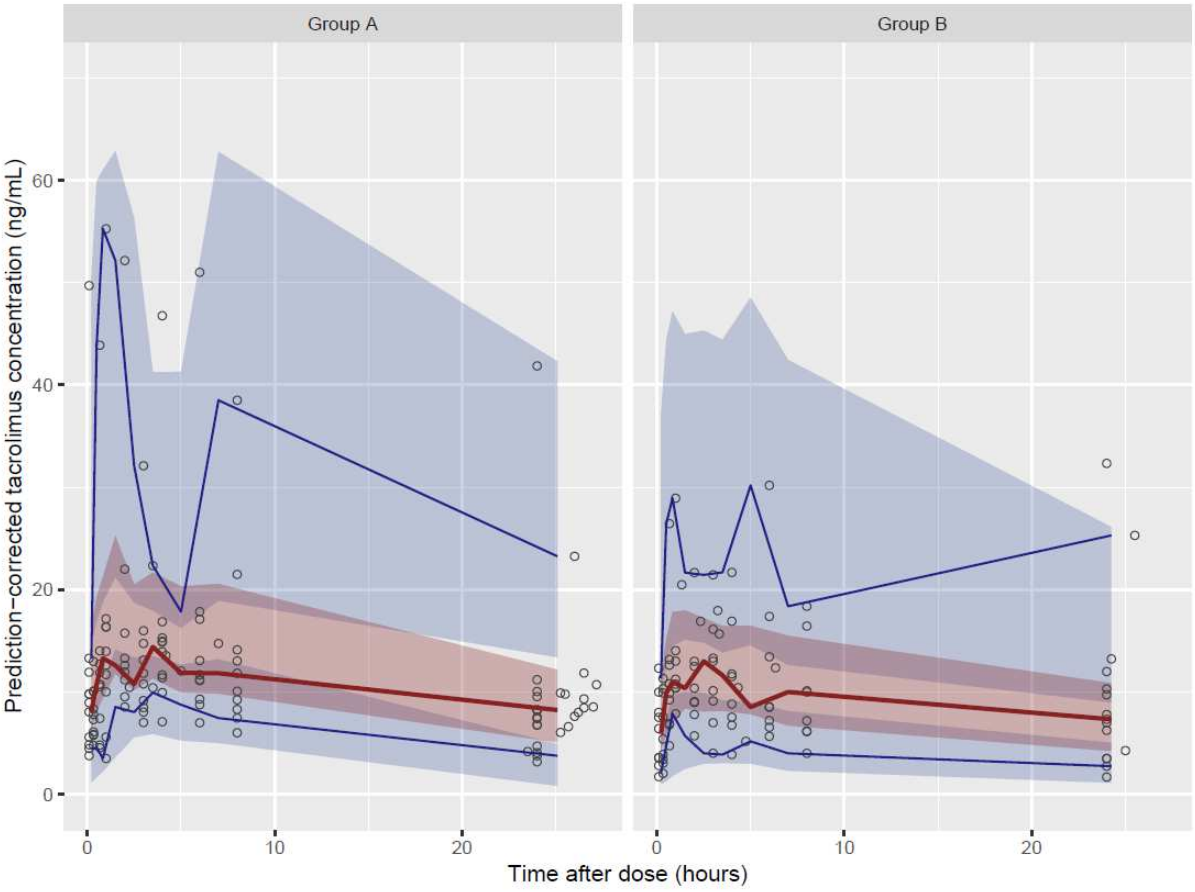
Supplementary Figure 1 (a) Correlation plots between individual absolute clearance (CL) obtained from the population approach and continuous covariates at the first pharmacokinetic occasion ($OCC_1 \leq 28$ days for group A and $OCC_1 \leq 105$ days for group B); (b) Correlation plots between individual absolute clearance (CL) obtained from the population approach and continuous covariates at the second pharmacokinetic occasion ($OCC_2 > 28$ days for group A and $OCC_2 > 105$ days for group B).

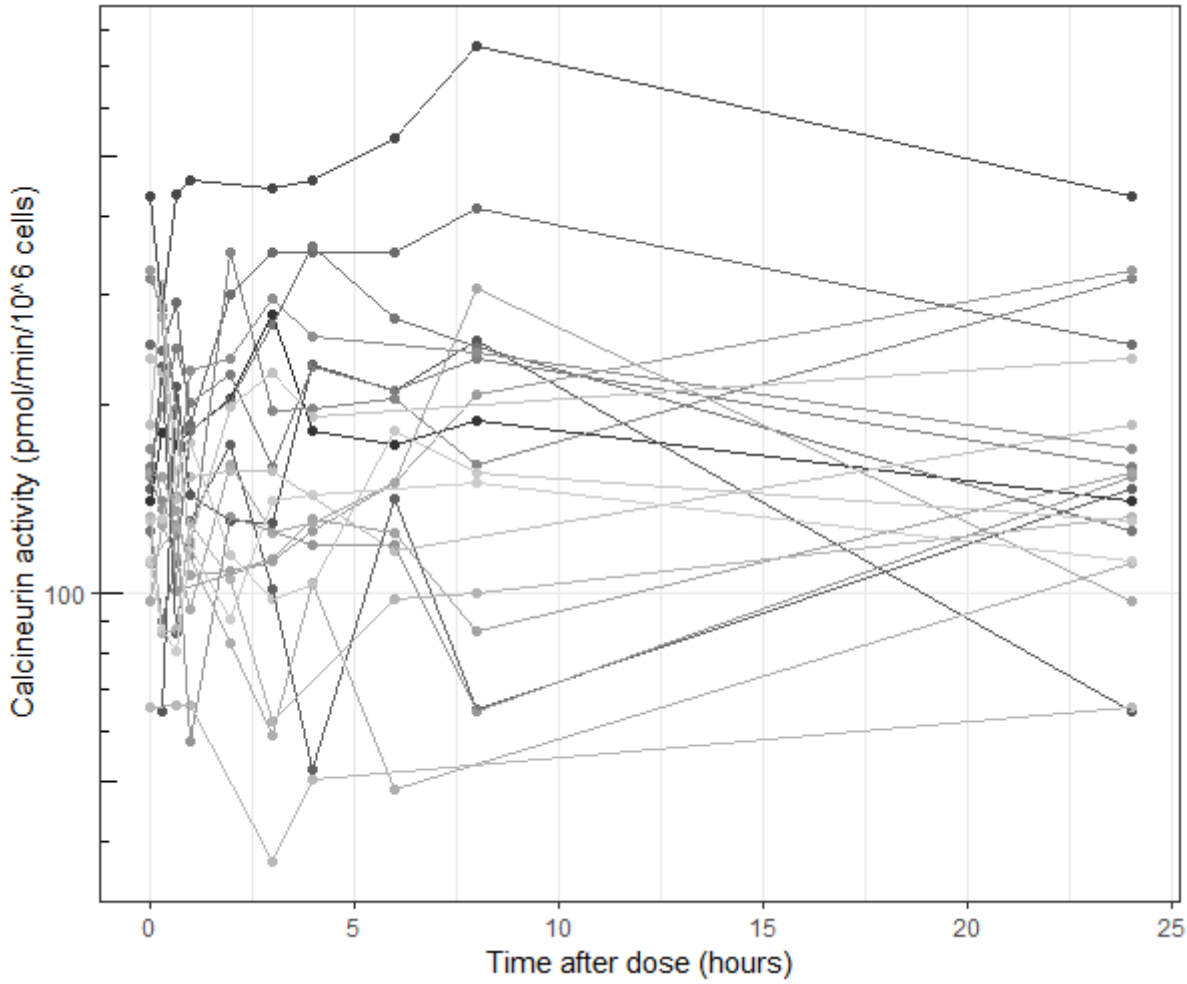
Supplementary Figure 2 Box-plots for individual absolute clearance (CL) obtained from the population approach and categorical covariates.


D1
Liver transplantation
Starting treatment with IRT
(Prograf®)
D5 D7
24h post-dose
0, 20, 40 min,
1, 2, 3, 4, 6, 8, 24 h
D30
24h post-dose
D90
24h post-dose
D180
24h post-dose
Group A (n = 12)
D7
Conversion
to PRT
(Advagraf®)
D14
Extensive PK
sampling
Calcineurin
activity
D90
24h post-dose
0, 20, 40 min,
1, 2, 3, 4, 6, 8, 24 h
D180
24h post-dose
Group B (n = 12)
D90
Conversion
to PRT
(Advagraf®)
D104
Extensive PK
sampling
Calcineurin
activity
Tacrolimus concentration
Calcineurin activity


Group A
Group B
Tacrolimus concentration (ng/mL)
20
10
0
5
10
15
20
25
Time after dose (hours)


DV (ng/mL)
IPRED (ng/mL)
DV (ng/mL)
PRED (ng/mL)
CWRES
Time after dose (hours)
CWRES
PRED (ng/mL)


Group A
Group B
Prediction−corrected tacrolimus concentration (ng/mL)
0
20
40
60
0
10
20
Time after dose (hours)


Calcineurin activity (pmol/min/10^6 cells)
100
0
5
10
15
20
25
Time after dose (hours)

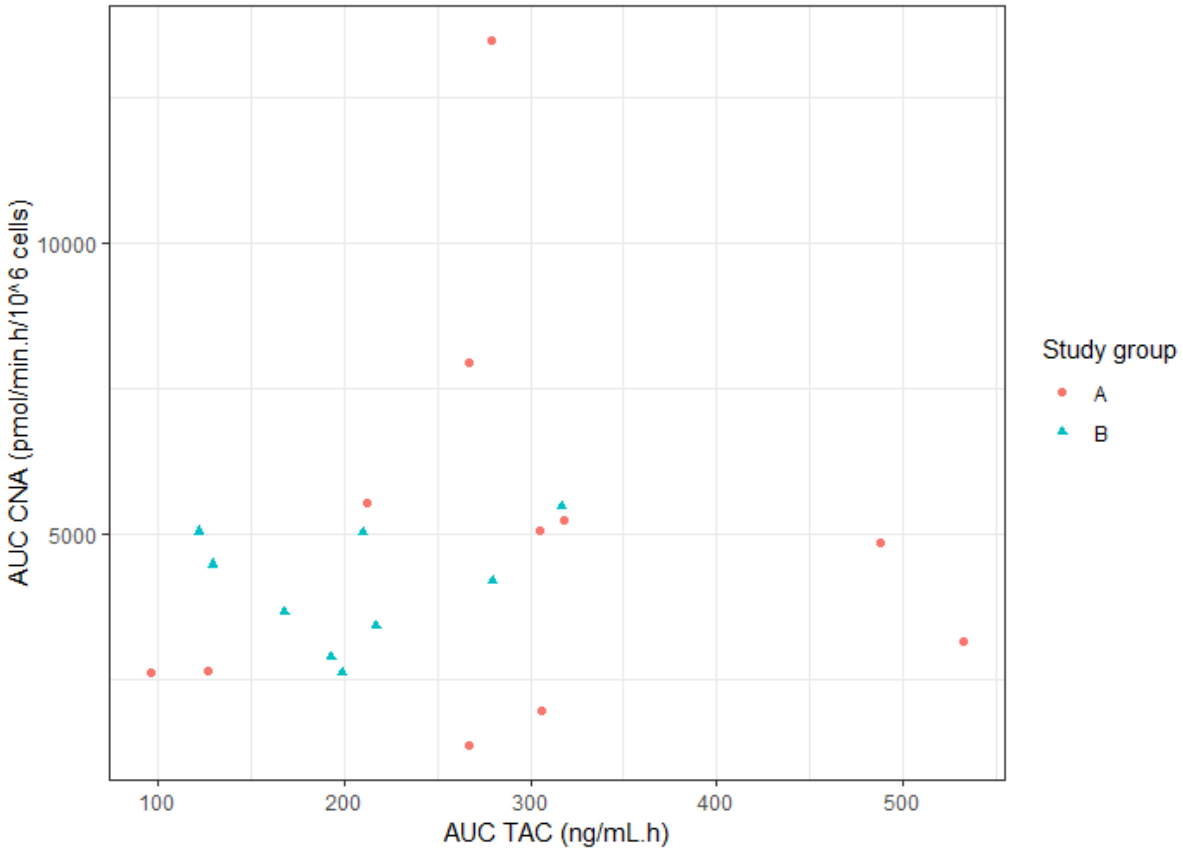

AUC CNA (pmol/min.h/10^6 cells)
10000
5000
100
200
300
400
500
AUC TAC (ng/mL.h)
Study group
A
B

Table 1 Baseline demographic and biological characteristics of group A and B. Results are presented as median [interquatile] or median (range).

	Group A (*n* = 12)	**Group B** (*n* = 12)
Sex (female/male)	3/9	1/11
Age (years)	57 [53-60]	59 [54-62]
Body weight (kg)	81 [69-86]	74.0 [67-81]
Lean body mass (kg)	15 [13-16]	15 [13-15]
Biological data		
Hematocrit (%)	31 [29-33]	38 [34-39]
GFR (mL/min)	105 [70-117]	82 [54-91]
AST (UI/L)	43 [16-69]	21 [17-25]
ALT (UI/L)	85 [26-125]	11 [9-26]
Albumin (g/L)	30 [28-34]	40 [36-47]
Bilirubin (µmol/L)	24 [17-54]	8.0 [7 – 11]
Tacrolimus therapy		
Median dose[a] (mg/day)	7.0 (2.0-20.0)	5.0 (2.5-12.0)
Trough concentration (ng/mL)[a]	8.5. [5.4-10.2]	5.6. [4.1-6.6]

AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; GFR, Glomerular filtration rate

[a] Median value at the time of extensive pharmacokinetic sampling

Table 2 Comparison of AUC_{TAC} obtained by non-compartmental analysis and population pharmacokinetic approach.

	AUC_{TAC} (ng/mL.h) [a] Geometric mean [CI95%]		p-value [b]	$AUC_{TAC\ Group\ A}$ /$AUC_{TAC\ Group\ B}$ Ratio of geometric means [CI90%]
	Group A (n = 11)	Group B (n = 9)		
Non-compartmental	234.5 [130.3 – 670.6]	231.0 [120.2 – 433.4]	0.77	1.01 [0.66 – 1.56]
Model-predicted	235.6 [139.6 – 598.7]	224.6 [117.6 – 421.5]	0.94	1.05 [0.70 – 1.57]
p-value [c]	0.90	0.25	NA	NA

CI, confidence interval; AUC_{TAC}, area under the tacrolimus concentration-time curve over the dosing interval; NA, not available

[a] Dose-normalized for median daily dose of 6.0 mg

[b] Wilcoxon unpaired test comparing non-compartmental and model-predicted AUC between group A and B

[c] Wilcoxon paired test comparing non-compartmental and model-predicted AUC for each study group

Table 3 Mean pharmacokinetic parameter estimates obtained from the final model and from 500 bootstrap runs with resampling.

Parameter	Mean estimate (%RSE) [shrinkage]	Bootstrap mean (95% CI)
k_{tr} (h^{-1})	2.19 (19.7%)	2.14 (1.37 – 2.92)
CL (L/h)	5.09 (8.2%)	5.11 (4.36 – 5.93)
V_c (L)	93.5 (41.0%)	86.9 (54.1 - 126)
Q (L/h)	42.0 (46.2%)	43.1 (20.2 - 71.9)
V_p (L)	135 (21.0%)	142 (88.4 - 196)
F (fixed)	0.23	0.23
Between-subject variability		
k_{tr} (CV%)	94.5% (22.1%) [13.3%]	80.6% (51.6 - 111)
CL (CV%)	34.7% (31.5%) [26.1%]	33.8% (13.5 – 48.4)
Q (CV%)	151% (37.6%) [34.6%]	120% (67– 170)
Between-occasion variability [a]		
CL (CV%)	39.8% (21.3%)	36.2% (22.8 – 46.6)
Proportional error (%)	19.8% (8.6%) [14.3%]	18.7% (16.0 – 21.1)

CI, confidence interval; CL, clearance; CV, coefficient of variation; F, bioavailability; k_{tr}, tansfer rate constant between transit compartments; Q, inter-compartmental clearance; RSE, relative standard error; V_c, volume of distribution of the central compartment; V_p, volume of distribution of the peripheral compartment

[a] occasions (OCC) defined as: OCC1 <= 28 days and OCC2 > 28 days (group A); OCC1 <= 105 days and OCC2 > 105 days (group B)